MEMORANDUM

BRISTOL LABORATORIES

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lederberg

TO J. Lein

C.C.

DATE December 22, 1955

SUBJECT New Antibiotics Screening Program - Consultantship Arrangement

Dear Joe:

It was very good to hear your recent word on the productivity of the program for screening for inducing agents. If nothing else, the procedure is at least turning up compounds of so-far unknown possibilities that would have been overlooked by the other procedures. It happens that I was preparing a more general perspective summary of my consultation work at the time that your last record arrived. I will defer that to other records and take this occasion to answer some of your specific questions about the induction program.

The instability of your active culture is certainly curious but the first question I would ask is whether it has cropped up again after repurification of the isolate. It is perfectly possible that it has no particular significance at all. If it really proves however that the ability to form this agent is an unstable property I doubt if the most likely explanation is to be found in terms of an unusually high mutation rate. It would appear more likely that the agent has a strong selective effect against its own parental type of cell. In that case once you have obtained a moderate amount of the purified material it may be feasible for you to select with this substance for colonies that would be more resistant to it and yet continue to be able to form it. It is of course quite possible that mutagenic action of the agent plays some role in the higher variability of the culture.

I suppose the next question that is going to come up is what to do with this type of agent. I have not heard any details at all from you about the technique of your anti-tumor screening program and I wonder if you have developed this to the point where I could have some knowledge of it. It seems to me rather important that a possible anti-tumor agent be tested on more than one system, although of course the Ascites tumors are the most likely candidates for a screening system. It is particularly encouraging that so small a proportion of your broths are proving to have inducing activity as this does make it likely that you are running into something a little bit new in the screening program. If your compound really has little or no antibacterial activity then perhaps it is going to prove to be of some considerable importance for theoretical investigations whether or not it is something that will earn Bristol any profits right away.

Apropos my earlier proposals, of course what I had in mind as the justification for looking for inhibition of inducing activity was the possibility of using this as a system for screening for antiviral activity. There is really not much that I can say about the possible superiority of this system over the more thoroughly worked over setups that Asheshov and others have been looking into with regard to the inhibition of phage lysis of bacteria. If after a brief introduction you should in fact find that a large fraction of your broths were showing this type of inhibition there would not be a great deal of point in continuing with the program. As far as I know, however,

nothing whatsoever is known about the mechanism of action of the inhibitors that the Botanical Garden group had uncovered and for that reason there is scarcely any theoretical basis on which to judge the possible advantages of another kind of screening program. I would be rather astonished myself if any general antiviral agent were to be discovered and in particular it would surprise me if such agents were picked up by the use of bacteriophage systems, but I really could not give you any sound theoretical reasons for that pessimism simply because we know so little about the possible mechanisms which may be involved. On this purely empirical basis therefore it would seem to me that it would be worth giving a summary sort of trial of a couple hundred broths for this kind of inhibition. It would be of particular interest to focus on those broths which had inhibitory activity on phage development without any great antibacterial action.

There are two possible points at which such an inhibitor might be expected to act in the development of lambda. One would be with regard to the induction process itself that the prophage might somehow be desensitized to the inductive effect. This I think would be unique for the lysogenic system and represent something that could not be picked up by looking at the ordinary infective systems. Whether this would in turn give you compounds that would be of interest in antiviral therapy is quite problematical but we are working so much in the dark that it seems to me even a few straws would be worth looking at.

The second point where an inhibitor might be acting would be in the growth of the activated phage itself and in this respect one would anticipate there would be no great difference between the lysogenic system and the lytic systems which Asheshov has examined. However, just because lambda is a temperate phage, not to mention the fact that it is simply a different phage from the systems which Asheshov had been using, I am not sure how far one should apriori extrapolate from his results. If my understanding is correct, very few antibiotic broths have been found to have any detectable activity against animal virus systems. In the circumstances it would seem to me worthwhile to make at least a trial to see whether anti-lambda induction activity is as prevalent as the anti-phage activity of the Botanical Garden group or whether on a purely empirical basis this is not an equally unique system. It seems to me important however that any follow-ups on the therapeutic possibilities of anti-phage or anti-inductive compounds ought to be made on systems which have at least a chemical similarity to the bacteriophages, that is to say, they should be tried on DNA as well as RNA-containing animal viruses. I know too little about the kind of work that you may have already been doing or anticipating doing in this field to judge the efficacy of your possible follow-up trials. I realize that the screening that I am suggesting is one of the chancier things that I would have any occasion to propose to you and I certainly would not press the matter if you consider the preliminary routine more expensive of time or money than any possible benefits that you can envisage. There is only the long-chance that the lambda system may prove to be rather different from the other phage systems that have been examined.

This is the kind of thing of course that it would be very much more appropriate to discuss personally and I am sorry to have to be in a position of putting you off so frequently about a possible visit. My work here is piled so high just at the moment that I can't see immediately when it will be possible for me to come but I will make every effort to find a date when I can do it, perhaps in mid-January or thereabouts. I would recommend that we not count on this as far as the exchange of information or consultation is concerned until I tell you just when such a trip actually would be possible. If it would be feasible to manage this so that we could consult, say on a

Saturday, that would give me much greater leeway in trying to plan the trip. Unfortunately I have had some very pressing personal obligations which have taken a little of the time that I might otherwise have been able to extract from my laboratory duties. Is there any chance that you or Felix or both of you would be interested to travel yourself and visit us in Madison? As long as I can keep up my lab routine with an hour or two's attention I would be able to discuss all of these matters with you at great length if there would be any possibility of your coming here. I won't extol for you the Wisconsin climate in the wintertime although I suspect it is not greatly different from what you are experiencing yourself at the present time.

On re-listening to this record, I realize that I had left out one point in possible mechanisms of inhibition and that is that an agent might prevent the lysis of a bacterium even though it had grown a full quota of phage. If this were the case, the infective center ought still to be detectable as such after the agent is diluted away and this would be a somewhat simpler thing to set up for the lysogenic response than it would be for ordinary phage infective bacteria. I will give the whole question of antiviral possibilities more thought and tell you what I can find out in due course.

Before I forget, I should caution you against the possibility of picking up hydrogen peroxide as an inducing agent. Since this is possibly going to be extracted by organic solvents and since hydrogen peroxide itself has been reputed to have strong inducing activity, it is something to look out for. If you have any reason to suspect peroxide, I suppose the easiest way of ruling it out would be its inactivation by catalase.

I would like to go on now to a more general set of topics. My understanding of my position with you, Joe, is that I am supposed to try to keep as much of a general perspective as I can and help to remind you of general issues at some intervals in order to help you and myself from becoming too much preoccupied with immediate details. For that reason I have gone over notes of our previous discussions and there are a list of topics that I think ought to be kept alive whether or not any immediate consideration is given of them.

1. What are antibiotics? I won't go over any details of previous discussions which would be somewhat obvious. The question I find myself left with is on what basis should Actinomycetes be unusually favorable sources for new antibiotics and as a corollary is this presumption really true or should more attention be given to other organisms as sources of <u>new</u> materials?

2. What is the status of the operational evaluation of the screening program? At what stages are different percentages of cultures eliminated in terms of the flow sheet? These are the kind of data that I would find very useful in trying to perfect further criticisms of the existing procedures. What are the means of classifying antibiotics to be sure of their identity excluding duplications etc. or is this a problem which is outside the Microbiology group? Is the renewed emphasis on low-yielding cultures working out in any reasonable fashion? And in order to evaluate any part of the program at least some arbitrary statement has to be made concerning the relative cost of each stage of screening. Without an estimate, even an arbitrary one, it seems to me impossible to try to reconstruct the program on any rational basis.

3. We have had some mention of screening compounds for usefulness as adjuvants rather than as primary antibiotics. Has anything more come of this?

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4. I am still somewhat concerned about the proportion of colonies which are stated to fail to grow on transfer from the original soil inocula. Can you remind me again what the plating media are for these initial isolations? I am concerned with the question as to whether these media would allow the development of auxotrophic species of Actinomycetes. I realize that most Actinomycetes grow well on a minimal medium without supplementation but any emphasis on finding new materials ought to pay some attention to the possibility of Actinomycetes with unusual nutritional requirements. It might even be worthwhile considering whether to select again vigorous prototrophic forms by some modification of the penicillin selection method for example as a means of looking for unique auxotrophic forms. I realize the complications that you will have by way of developing selective media with such a procedure. In our lab we have found Mycostatin to be a very useful adjunct in preventing the growth of mold contaminants in our Actinomycete plates. Of course you run the risk of inhibiting some Actinomycetes but this is something worth considering.

5. Looking over the flow sheet, I see there is some reference to contaminated broths which I gather are distorted even though they may show considerable activity. Would it be worthwhile to take such broths to find out what the source of antibiotic activity is? You may either miss an antibiotic that could be obtained by reisolating the Actinomycete or even conceivably this would be one way of stumbling onto an antibiotic produced by a bacterium. At any rate I would think that if a broth has substantial activity it ought not to be simply discarded because it appears to be contaminated. Does contaminated mean turbid? If so, I think there is the question whether in fact these are contaminated broths or whether you may not be running into some peculiar Actinomycete forms which are capable of giving a certain amount of turbid growth. In any event I would be interested to hear just what these contaminated broths are and why they are being discarded. Also, as concerns the flow sheet, the main criticism that I think I might make of the procedure is the failure to subject every fermentation broth that shows a trace of activity to a time series. It seems to me that the additional effort required to test whether substantial activity might not be produced over different ranges of time would be well-worth not dropping any culture that shows even a trace of activity in the first screening on a variety of media. I am also concerned about the failure of replication, that is to say, the indicated occurrence of cultures which gave satisfactory broths in the first screening and which "failed to hold up" on the second screening. Have you any ideas for the reasons behind these discrepancies? I emphasize minutiae of this kind because it seems to me that the small laboratory is actually in a better position to trace down this kind of distraction than is the large one which is set up on an even more involved routine basis and as you have repeatedly emphasized to me you have to find the optimal basis for your competitive position as aginst the larger setups.

6. The flow sheets refer to a mutation program but I have heard practically nothing from you about what is actually done here and would like to know if you are pursuing this avenue to speak of. It seems to me that a more routine application could be made of the mutation program to try to improve the activities of some of the marginal producers that you don't quite want to discard during the intermediate screening stages. That is to say, on your flow sheet #1 near the bottom center where it says further fermentations until active or discarded, it seems to me that these would be unusually apt candidates to put into the mutation program. Once again it would seem to me that it is in this group that you are most likely to find

6. agents which would be overlooked by other workers.

7. Since the purpose of a screening program is to start with a maximum of genetic heterogeneity in the first place I wonder if it would not be worthwhile trying to increase that initial heterogeneity by a rather gross treatment of your samples before they are even plated with mutagenic agents. For example, it might not be completely unworthwhile to include such an active mutagen as azaserine in the medium in which the samples are held just prior to plating or even in the plating media themselves. This is kind of a wild shot but it would be one means of increasing the diversity of types on which your routine screening is going to be exercised.

8. As another possible application of mutagenic or inducing agents, I am reminded of the finding that the bacteriocins, like pyocinase, are released from bacteria under conditions which are closely analogous to the induction of bacteriophage. This might have some bearing on the improvement of yields of antibiotics.

This was just a small interruption now, Joe. There was a little fire in the room next door but after 10 or 15 minutes of scurrying around the thing was put under control so I'll try to get back to order now.

Perhaps the inducing antibiotic that you've just picked up would be worth using in a trial of this principle, first because it has at least a low antibacterial activity and would not confuse the test and second because of the presumption that it does have some effect on the Actinomycete. At least if you are constructing a variety of media with various inhibitors designed to help provoke the production of antibiotics, I would suggest including in such a series compounds with known inducing activity.

9. One of the most familiar facts of screening for antibiotics is the very high animal toxicity which most of these compounds demonstrate and which of course makes them useless for therapeutic purposes. Have you any notion why so many of these agents should be so toxic? Perhaps this reflects a general protoplasmic toxicity for which the screening program would be of course selective but it's a little surprising in a way then that the Actinomycetes themselves should fail to be susceptible to agents whose common bond of toxicity will then be some bacteria and some mammals. Even if this is true it suggests the possibility that there may be some species specificity in the toxic action of some of these therapeutically useless materials which raises the question of whether you thought there might be other product applications for these toxic agents. I had in mind the possibility of one use as pesticides of some kind for example in the elimination of rodents, a use which probably would require demonstration of a certain amount of selectivity or applications such as that of poisoning of lakes in order to remove fish. Would it be too far fetched to propose some consideration of toxicity tests on, say for example, gold fish, as part of the program because I suspect that there might be a market for any <u>extremely</u> toxic agents for such diverse purposes, and you might get a lead on some activity of toxic effects. Now I appreciate that antibiotics may turn out to be too expensive in terms of cost of production to compete with other agents but this is a possibility at least to be kept in the back of one's mind and the particular question where there may be some species specificity and toxicity which would mean of course that using, say the mouse, as a screening agent may not tell you all that you want to know about man. Somewhat amusedly I would also be led

9. to inquire whether some of your products might not have other kinds of toxic activity and might for example furnish a cheaper and still marketable substitute for colchicin. I don't know how large the market for a drug of this kind is but if you look at the price which is being charged for it, I think you can see that it would not take a tremendous market to make it worthwhile to produce an effective substitute. I'm sure I don't have to propose how one might go about screening for this type of activity. It would involve I imagine for the roughest tests looking for the formation of C tumors in onion roots. I would keep in mind that compounds with C-mitotic activity have been proposed also as anti-tumor agents and if you are disposed to wild flings it might not be completely worthless to give some consideration to screening for that sort of activity in relation to tumor therapy as well as for a polyploidizing agent.

10. A decisive stage in your screening program is the initial picking of the colonies which are going to be followed in more detailed study. Perhaps the time that I spent during my last visit was not entirely typical but I have the feeling that this particular phase of the work was perhaps not as closely tied in with the rest of the program as perhaps it could be, and that it would be worthwhile considering whether a great deal of professional attention should be concentrated on this specific phase of the screening program.

11. Do you happen to know whether chlortetracycline is the yellow pigment which is produced by Streptomyces aureofaciens? If this is so, then it seems to me that it would be useful as an aspect of the mutation program to look into the pigmentary variation of this Actinomycete. I had in mind that the occurrence of mutants which still had appreciable antibiotic activity, although lacking the original yellow pigmentation, might furnish a technique towards the discovery of still further antibiotics related to tetracycline. I will be mildly surprised should this be a feasible proposal you have not already explored from a number of angles. If this is the case, I would appreciate for the purpose of my own general background being referred to any literature or other information on that particular point.

12. We have had some discussion about screening programs for the detection of biological activity other than bacterial inhibition. These have included virus induction in the K-12 system which seems to be very well under control, the inhibition of virus development which is discussed in more detail on the other side of this record and a proposal that you brought up on testing for the production of small colony variants in yeast. On this last point we have been in some contact with Ephrussi for our own purposes and I am still not very happy about the applicability of the red adenine-requiring mutant for a simplified procedure. Ephrussi has sent us cultures in which this effect is supposed to pertain but evidently a certain amount of manipulation of the medium is required in order to insure that the red adenine stock is allowed differentiation of normal and petit. Ephrussi writes that it is necessary to use a very low sugar medium in order to prevent the petit types from showing the red color as well. In fact, since we are trying to adapt almost precisely the same system for our own purposes it seems to me that it would be most practical for you to wait on this until we have looked into this ourselves unless of course you have much more encouraging and detailed information from other sources. I am willing to predict that you will find a rather close but not an absolute correspondence between the agents which are detected as inducing agents and those which will show up by this particular system which is not to say that it would not be worthwhile to go ahead with the yeast provided that it does not represent too

12. much of a distraction to your other efforts. The main question is what to do with the further evaluation of agents which are picked up by these biological activities. Any possible virus inhibitors would of course be worth studying for their chemotherapeutic effects in eggs, in mice etc. and I think, unless too many of them turn up, they should be given a fairly comprehensive examination with a variety of animal viruses. I have already asked you the question as to the status of the tumor agent screening program.

13. A little while ago you asked me if I had any notions about properdin. I have asked some of my immunological colleagues about this. There evidently is not very much exact information on what properdin is or what it can do. It can be best summarized by calling it a component of complement and in that respect nothing very remarkably new. I could not find out anything that would suggest the desirability of going into this kind of program unless you meant to do it in quite a large way as a major step.

14. Apropos of our earlier discussions on bacterial replacement therapy to mitigate complication of the use of broad spectrum antibiotics, I saw an advertisement in a New York paper last week which advertised the use of acidophilus milk "to counteract the dangerous side-effects of antibiotics". I am not clear whether this is intended to be used in conjunction with antibiotic therapy or as a means of hastening recovery from it. Have you heard anything about this? I have not seen any technical medical literature that would help to justify the use of this particular preparation. It does however suggest an angle, namely, instead of feeding something called Serratia as a separate medical article the development of appropriately antibiotic-resistant strains of organisms used in the manufacture of milk, sour cream, cheese etc. which might at least carry not so bad an odor to the medical profession in their use as biological therapeutics. I am still concerned about the possible dangers of introducing any new organisms into the situation until there has been a very thorough study of the possible perils. To feed acidophilus of a standard variety to a person receiving antibiotic therapy is one thing and if the Lactobacilli are still susceptible to the antibiotic it may be expected to do very little good at all. If we really wanted to accomplish something by using antibiotic-resistant Lactobacilli to start with, we run the risks that are associated with their introduction in essentially pure culture into the human gut. I will be very much interested to hear the immediate outcome of the experiments that you said were being started with things like the Serratia organism that I had sent to you. In any event there does seem to be some possibility that should be looked into of modifying that program to the development of antibiotic-resistant strains of microorganisms which are already customarily used in the food industries. Of course from the point of view of the Bristol Company one difficulty with that is that is does not directly lead to further product development but it perhaps is something that you could work on together with some of the dairy industries or some people of that kind. In any event I am sure that you would have some interest in any procedure which would help to prove the suitability of antibiotics and chemotherapy.

This is about all I have to say by way of immediate summary. The only thing I would repeat for purpose of emphasis and just because it is a rather concrete thing concerns this detail on flow sheet #1 in the screening program. In one sample of data that you gave me there was as I recall a split of so-called 75 non-active to 91 active broths in the first screening. Of these 91 active broths in this particular series, 16 were rejected for one reason or another and 75 were given a time study. That is to say,

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about an equal number were discarded as being "non-active" or being active. However, as best I can make out the so-called non-active organisms did include types which had shown very small degrees of activity. I am sure that you're not doing a mutation program on all of them. My immediate suggestion is that every culture which has shown any activity whatsoever in any broth ought to be given a time study and in fact it might be worthwhile giving time studies even to the apparent negative cultures. Now that means of course that you'll have to choose your media somewhat blindly. The other situation was that the main source for cultures to be used in the mutation program or perhaps not the ones which are completely inactive but the ones which might otherwise be discarded for lack of sufficient activity to be rundown biochemically.

As per custom, please let me know if I have been clear enough about any particular point. The reason that I wrote to Dr. Gourevitch rather than use the Voicewriter was simply that I had the typewriter handy and not the department machine at that particular time.

Best wishes to you and Pat.

Yours sincerely,

Joshua

JL:jlg